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Research Article

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In vitro egg hatchability inhibition effect of *Albizia gummifera, Phytolacca dodecandra*, and *Vernonia amygdalina* against natural infection of ovine GIT nematodes

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ABSTRACT

An *in-vitro* experiment was carried out to evaluate the egg hatching inhibition effect of three herbal plants, namely; *Albizia gummifera*, *Phytolacca dodecandra*, and *Vernonia amygdalina*. The leaves of these plants were collected; air dried and powdered with pestle and mortar, and then hydro-alcoholic extraction was performed and in measuring the percentage yield, *P. dodecandra* givesbetter yield (15.34%). Crude extract of these plants were evaluated for egg hatchability assay at different concentrations (3mg/ml, 5mg/ml and 10mg/ml) for each plant and the experiment was replicated five times. Ivermectin (0.1ml/ml) was used as positive control. Amon AQ1:Kindly provide any one corresponding author email id g the plants, the crude extracts of *P. dodecandra*had better activity that achieved maximum (100%) egg hatch inhibition at concentrations of 5 mg/ml while *V. amygdalina* and *A. gummifera* induced complete inhibition at concentration of 10 mg/ml after 48 hours of exposure. All the three plant crude extracts were inhibit egg hatchability significantly ($p< 0.05$) as compared with the negative control but the inhibition among them were not significantly different in the effect. In conclusion, this study revealed that all of the three plant extracts have high inhibition potential on the hatchability of gastrointestinal nematode eggs. More detailed study on *vivo* anthelmintic effects of these plants with different extraction methods and phytochemical screening should be done.

KEYWORDS: *Albizia gummifera;Phytolacca dodecandra;Vernoniaamygdalina*

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INTRODUCTION

Helminth infections are among the most economically significant diseases of small ruminants worldwide [1]. In the Developed world, the greatest impact parasitic diseases is probably found in the costs of control, while in Developing one, the impact lies in productivity losses [2]. Ethiopia ranks third in numbers of sheep and goats among Africa nations and ranks eighth in the world [3]. However, gastrointestinal nematode infection has greatest impact on the survival and productivity of sheep and goats [4]. The greatest losses associated with nematode parasite infections can be direct due to a drop in production and other parasite-related penalties [5].

Control of nematode parasites has been based on the use of commercial anthelmintic drugs. However, due to repeated use of these drugs, resistance against drugs is an issue [6, 7]. Therefore, investigation of alternative to commercially available drugs like medicinal plants has paramount importance. In Ethiopia, studies are less to scientifically explore, evaluate, document and promote these medicinal plants in the country for their claimed activities [7, 8].

The aim of this study was to assess the *in vitro* egg hatchability inhibition effect of *Albizia gummifera*, *Phytolacca dodecandra*, and *Vernonia amygdalina* against natural infection of ovine GIT nematodes.

MATERIALS AND METHODS

Study Area

The study was conducted in Gondar University veterinary laboratory, North Gondar Zone, Amhara Regional State, Northwest Ethiopia. Gondar is located 738 km North West of Addis Ababa

which is found between geographically coordinates of 12° to 40° N longitude and 37° to 45°E latitude with an altitude range of 1800-2200 meter above sea level. The ranges of maximum and minimum temperature vary between 22-30.7°C and 12.3-17.1°C, respectively. The area receives average annual precipitation of 1000mm [9].

Plant Material Collection

Leaves of *Albizia gummifera; Phytolacca dodecandra* and *Vernonia amygdalina* were collected in Gondar University Atse Tewodros campus. The collected plant parts were identified plant taxonomist and allowed to air dried at room temperature, powdered using pestle and mortar, and stored until extraction.

Plant Extraction Method

Hydro-alcoholic extraction was performed as explained previously [7].

Collection of Parasitic Eggs

Faecal pellets were collected from the rectum of naturally infected sheep which was maintained in heavily contaminated pastures and was untreated for at least six months. The sample was placed in a sampling bottle and transported immediately to Gondar University veterinary parasitology laboratory. For further analysis, the parasitic egg was collected and the number of eggs per ml was determined based on the brief explanation of Getachew *et al.* [7].

Egg Hatch Inhibition Assay

The Egg Hatch Assay was conducted according to the World Association for The Advancement of Veterinary Parasitology guidelines [10] as decribed by Eguale *et al.* [8].

Data Management and Statistical Analysis

The experimental data was recorded in Excel spreadsheet subjected to descriptive statistical analysis to derive mean and standard deviation. The hatchability inhibition effect of the extracts of the plants was compared by Analysis of Variance (ANOVA). Further individual mean significant difference was calculated by using post hoc test LSD (Least Significant Difference test) by using SPSS software.

RESULTS

Among the plants used in this study, *P. dodecandra* givesbetter yield (15.34%) as compared to A. *gummifera* and V. *amygdalina* (Table 1).

After 48 hours, *in vitro* exposure of parasitic eggs to different concentrations of hydro-alcoholic plant extracts produced high egg hatch inhibition proportions that were dose-dependent as compared to the negative control. The extracts showed good hatchability inhibition activities against eggs of ovine GIT nematodes. Maximum concentration (10 mg/ml) induced 100% egg hatch inhibition (Table 2). Extract of *P. dodecandra* induced 100% egg hatch inhibition at its second minimum concentration

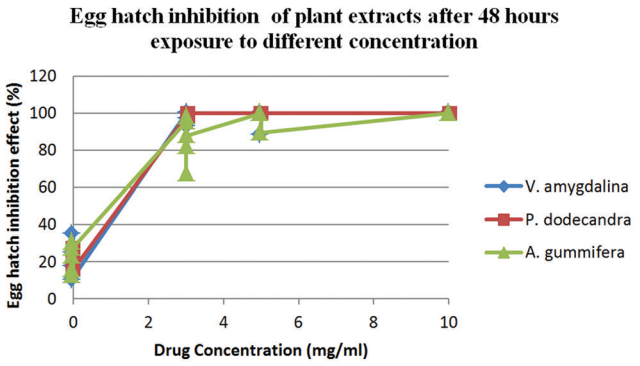
Table 1: Percentage yield of different plants extracts

Plant species	Plant parts used	Extract type	Percentage yield (%) (w/w)
A. gummifera	Leaves	Hydro-alcoholic	11.67
P. dodecandra	Leaves	Hydro-alcoholic	15.34
V. amygdalina	Leaves	Hydro-alcoholic	14.23

of 5mg/ml while V. *amygdalina* revealed 98.62% inhibition at both 3mg/ml and 5mg/ml concentrations. Among all plant tested, extract of A. *gummifera* was relatively the weakest that induced 86.14% egg hatch inhibition at concentration of 3mg/ml and it resulted97.9% egg hatch inhibition concentration of 5mg/ml. The positive control (ivermectin) exhibited relatively good result and induced 100% egg hatch inhibition at a concentration of 0.1ml/ml.

SE* = standard error

The result of this revealed that all plants induced egg hatch inhibition statistically significant differences between negative control and different concentrations all plant extracts but there were no significant difference between different concentrations and positive control. Among all plants different concentrations, only A. *gummifera* show statistically significant difference at its 3mg/ml (p=0.041).



DISCUSSION

This research was conducted *in vitro* due to the reason that *in vitro* technique had advantage to evaluate anthelmintic activities of claimed medicinal plants over *in vivo* techniques due to simplicity and cost effectiveness of this technique [11].

The findings of the present study revealed that all plant extracts exhibit anthelmintic effect at different concentrations and the efficacy of extracts increased with increasing concentration. The report of Biftu *et al.* [12] indicated that the sheep with mixed natural infections treated with A. *gummifera* exhibited significant faecal egg reduction and the this study confirms the anthelmintic property of this plant extract by evaluating the *in vitro* effect of this plant against mixed infection of sheep [13]. However, the finding of this research even in its minimum concentration (3mg/ml) is far from the report of Chufamo *et al.* [14] that 70% acetone extract of A. *gummifera* induced only 48.9% *in vitro* egg hatch inhibition against *H. Contortus.*

Table 2: Mean percentage inhibition of egg hatch of ovine GIT nematodes after 48 hours exposure to different concentration of plant extracts (mg/ml)

Mean±SE* percentage inhibition at different concentration					
Plant type	Negative control	3	5	10	Positive control
A. gummifera	21.7±3.4125	86.14±5.459	97.9±2.1	100±0.00	100±0.00
P. dodecandra	21.64±2.7458	99.4±0.4	100±0.00	100±0.00	100±0.00
V. amygdalina	19.38±4.5587	96.42±1.68	97.64±2.36	100±0.00	100±0.00

SE*=standard error

The results of this study showed that *P. dodecandra* has higher anthelmintic potential. According to Mohammed *et al.* [15], *in vitro* anthelmintic effect of *P. dodecandra* resulted 99.4% inhibition of egg hatchability at a concentration of 0.1 mg/ml which is compatible with the result of this study even if the concentration is different and 68.1% of adult mortality of *H. contortus* at concentration of 4 mg/ml. The report of Innocent and Deogracious [16] supports this result idea that *P. dodecandra* exhibited 100% ascaricidal effect at a concentration of 10mg/ml after 48 hours of *in vitro* test.

The results of *V. amygdalina* in this study support the trials by the traditional healers that these plants treat helminth infections in livestock [17]. The good anthhelmintic potential of *V. amygdalina* observed in this research is supported by the report of Adediran and Uwalaka [18] that the plant had 100% faecal egg count reduction effect against goat's helminth parasites. However, the report of Nalule *et al.* [17], and Innocent and Deogracious [16] is far from the findings of this study which states that this plant reveals only 64% faecal egg reduction count against mixed infection of goat's worm and kills 50% of the parasite at 6mg/ml respectively. Moreover, this finding contradicts with the result reported by Alawa *et al.* [19] and Sawleha [20] that states *V. amygdalina* did not show any significant *in vitro* anthelmintic activity at concentrations up to 11.2 mg/ml.

CONCLUSION AND RECOMMENDATIONS

In this study, extracts of both study plants (*V. Amygdalina and A. gummifera*) have shown promising *in vitro* activity against eggs of ovine GIT nematodes. Depending on the egg hatch inhibition efficacy of these plant extracts, *V. Amygdalina* more potent than *A. gummifera*. This study supports the idea of pastoral communities and traditional healers in use of plant anthelmintics and to justify their potential of traditional and ethno-veterinary use of medical plants. From our results, below recommendations can be done:

- These plants should be tested with other types of extraction methods and further should be evaluated *in vivo*.
- Other different parts (flowers, fruits, stems and roots) of those plants should be evaluated for their anthelmintic effect *in vitro* as well as *in vivo*.

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